EXTENDED REPORT

Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage

O Meyer, C Labarre, M Dougados, Ph Goupille, A Cantagrel, A Dubois, P Nicaise-Roland, J Sibilia, B Combe

Ann Rheum Dis 2003;**62**:120–126

See end of article for authors' affiliations

Correspondence to: Professor O Meyer, Service de Rhumatologie, Hôpital Bichat, 46 rue Henri Huchard, 75018 Paris, France; olivier.meyer@bch.ap-hop-paris.fr

Accepted 7 June 2002

Objective: To study the value of antibodies to citrullinated proteins/peptides for predicting joint outcomes in patients with recent onset rheumatoid arthritis (RA).
Methods: 191 patients with RA onset within the past year were followed up prospectively for five years. Serum samples obtained from 145 patients at baseline before disease modifying antirheumatic drug treatment were examined using three anticitrullinated protein/peptide antibody assays: antiperinuclear factor (APF) by indirect immunofluorescence (IIF), antikeratin antibodies (AKA) by IIF, and anticyclic citrullinated peptide (CCP) antibodies by enzyme linked immunosorbent assay (ELISA). Radiographs of the hands and feet taken at baseline and after three and five years were evaluated using Sharp scores modified by van der Heijde.
Results: Anti-CCP ELISA was positive in 58.9% of patients. APF/anti-CCP agreement was 77%. The likelihood of a total Sharp score increase after five years was significantly greater among patients with anti-CCP antibodies (67%; odds ratio (OR) 2.5; 95% confidence interval (95% CI) 1.2 to 5.0) or APF (57%; OR 2.4; 95% CI 1.2 to 4.9) but not rheumatoid factor (RF; OR 0.7; 95% CI 0.3 to 1.5). Mean values for radiographic damage, erosion, and joint narrowing scores at the three times were significantly higher in patients with anti-CCP or APF than in those without. AKA did not significantly predict radiographic damage. In separate analyses of patients with and without RF, anti-CCP or APF was better than RF for predicting total joint damage and joint damage progression after five years.
Conclusion: Antibodies to citrullinated proteins/peptides determined early in the course of RA by APF IIF or anti-CCP ELISA are good predictors of radiographic joint damage. Further studies of clinical, laboratory, and genetic parameters are needed to improve RA outcome prediction in clinical practice.

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterised by progressive erosions and cartilage destruction. The current therapeutic strategy uses increasingly aggressive regimens early in the course of the disease. Thus, early diagnosis is crucial. The early diagnosis of RA relies chiefly on clinical manifestations and serological markers such as rheumatoid factors (RFs) or anticitrullinated protein/peptide antibodies. In contrast, the 1987 American College of Rheumatology (ACR) criteria are rarely met during the first few months of the disease.[1]

For detecting antibodies to citrullinated proteins/peptides, at least three tests have been reported. In 1964, Nienhuis and Mandema described antiperinuclear factor (APF),[2] which specifically targets an antigen (perinuclear factor) present in the keratohyaline granules surrounding the nucleus of human buccal mucosa cells. APF is found in sera from 40–90% of patients with established RA,[3] but is less common in early RA (27–38%).[4 5] APF can be detectable before the clinical onset of the disease and is considered a good diagnostic marker for RA.[6] In 1979, Young and coworkers identified antikeratin antibody (AKA) using indirect immunofluorescence (IIF) to examine unfixed rat oesophagus cryostat sections.[7] Serum AKA is present in 37–59% of patients with RA and is sometimes detectable before the clinical onset of the disease. Finally, recent studies by Schellekens *et al* and Girbal-Neuhauser *et al* have shown that APF and AKA specifically bind to substrates containing modified citrulline.[8 9] Filaggrin contains a large amount of citrulline. This amino acid may also be abundant in vimentin, Sa antigen, and fibrin from synovial joint fluid of patients with RA.[10 11] An enzyme linked immunosorbent assay (ELISA) that detects antibodies to cyclic citrullinated peptide (CCP) has been developed to simplify the determination of antifilaggrin antibodies. The group that developed this assay reported more than 85% specificity and at least 60–75% sensitivity for RA.[12] However, in another study, the proportion of patients with early RA with a positive anti-CCP ELISA was only 40% overall: 54% in rheumatoid factor (RF) positive patients, and 14% in RF negative patients.[5]

Among serological markers, RF has been recognised as an important predictor of more severe structural joint damage.[13–19] Whether citrullinated protein/peptide antibodies are useful for predicting outcomes in RA remains a matter of debate. The presence of APF was associated with more severe erosions in the hands in one of our studies.[13] Other groups have reported a correlation with erosions[20 21] or with functional class III disease.[22] Paimela and colleagues found greater disease activity among patients with early RA with antifilaggrin antibodies detected using an ELISA with purified human filaggrin.[23] The same group obtained similar results for AKA detected by IIF.

Abbreviations: ACR, American College of Rheumatology; AKA, antikeratin antibody; APF, antiperinuclear factor; CCP, cyclic citrullinated peptide; CI, confidence interval; CRP, C reactive protein; DMARD, disease modifying antirheumatic drug; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; IIF, indirect immunofluorescence; OR, odds ratio; RA, rheumatoid arthritis; RF, rheumatoid factor

Van Jaarsveld and coworkers found that a positive result for APF or anti-CCP ELISA early in the course of RA was associated with more severe radiographic damage after three years of follow up in both RF positive and RF negative patients.[24] Similar findings have been obtained by Westgeest and coworkers in patients with longstanding RA.[25]

AKA detected by IIF showed no correlation with joint damage progression in most studies.[26] However, correlations have been found between AKA and greater disease activity as evaluated by the Ritchie index, morning stiffness, and laboratory measures of inflammation such as the erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) level.[27]

In our study we used three methods to assay antibodies to citrullinated proteins/peptides in the first serum samples obtained from patients with recent onset RA and a negative history of treatment with disease modifying antirheumatic drugs (DMARDs). Our objective was to determine the value of these antibodies for predicting the development of joint damage during a five year prospective follow up.

PATIENTS AND METHODS

A cohort of 191 patients who met at least four 1987 ACR criteria for RA[27] and had a disease duration of less than one year were followed up prospectively for five years. None had received DMARDs at baseline. Serum samples obtained at baseline were stored at −20°C in aliquots of 1 ml. Numerous clinical, laboratory, immunogenetic, and radiographic data were collected and used to determine the disease activity score (DAS28), the swollen and tender joint counts, and the Health Assessment Questionnaire score.

Radiographic measurements

Radiographs of the hands and feet were taken at baseline and after three and five years. Three year radiographs were evaluated in the chronological order of patient inclusion by two independent observers who were unaware of the patient data. These observers used the Sharp method modified by van der Heijde.[14] For each patient, an erosion score, a joint space narrowing score, and a total damage score calculated as the sum of the first two scores were determined for the hands and feet; higher scores indicate worse outcomes. Firstly, we calculated the intraclass, intraobserver, and interobserver coefficients of correlation based on 30 selected pairs of radiographs of the hands and feet. These coefficients were consistently higher than 0.85, and no systematic differences were found for any of the scores. Then, for each of our 191 patients, we calculated the mean of the two observers' scores for erosions, narrowing, and total damage, at the hands and at the feet. To determine a cut off value above which any joint space narrowing would define individual radiological progression of RA unrelated to measurement error (that is, the smallest detectable difference), we calculated the mean of differences between two analyses as described previously.[28 29] To this end, we selected 30 pairs of radiographs of the hands and feet that were representative of the study group. For each score, we calculated the mean (SD) of the differences between the values given by the two observers. In accordance with OMERACT recommendations,[29] we defined radiographic progression as a radiographic score change greater than the upper bound of the 95% confidence interval (95% CI) of the relevant difference—that is, a change at five years of at least 4.1, 3.2, and 5.5 in the erosion score, narrowing score, and total score, respectively.

Citrullinated protein/peptide antibody determination

AKA

AKA IgGs were sought using IIF as described by Young *et al*,[15] with a few modifications.[30] Serum samples were diluted 1:10.

Table 1 Baseline characteristics of 191 patients with early rheumatoid arthritis

	Mean (SD) or No (% of patients)
Female (%)	140 (73)
Age (years)	50.5 (14.7)
Mean disease duration (months)	3.6 (2.6)
Pain (100 mm VAS)	57.5 (22.0)
Morning stiffness (min)	84.9 (79.4)
Ritchie index	17.5 (8.5)
Tender joint count	21.7 (10.5)
Swollen joint count	9.0 (5.9)
Nodules (%)	11 (6)
ESR (mm/1st h)	40.2 (28.5)
CRP (mg/l)	34.1 (43.2)
IgM or IgA RF positive (%)	139 (73)†
DAS	4.1 (0.8)
HLA-DRB1*04‡	86 (45)
HLA-DRB1*01	54 (28)
Number of DMARDs/patient	1.7

†IgM positive (%): 69%; ‡RA associated DRB1*04 genes: 0401, 0404, 0405, 0408.
VAS, visual analogue score; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; RF, rheumatoid factor; DAS, disease activity score; DMARD, disease modifying antirheumatic drug.

APF

IgG APF were detected using IIF on human buccal mucosa smears. Serum samples were diluted 1:40 and 1:80 to improve specificity. Serum samples diluted 1:80 that produced fluorescence of more than 10% of the cells were considered positive.

Anti-CCP ELISA

The ELISA was performed using a commercial kit (Immunoscan RA, Eurodiagnostica Arnheim, The Netherlands), according to the manufacturer's instructions. Patient serum samples were diluted 1:50 and were considered positive if the antibody titre was greater than 50 arbitrary units determined by dilution of a positive standard serum.

IgM and IgA RFs

IgM and IgA RFs were determined by in-house ELISAs. Plates were coated with purified human IgG (1 mg/ml) diluted 1:200. Patients serum samples were diluted (1:200 for IgM, or 1:50 for IgA). RF activity was shown using diluted horseradish peroxidase conjugated $F(ab)^2$ rabbit antihuman IgM (1:200), or IgA (1:2000) (sensitivity 90%; specificity 90%). Both ELISAs were considered positive if ⩾20 IU/ml.

Statistical analysis

The χ^2 test was used to examine concordance between the three anticitrullinated protein/peptide test results at baseline and to look for associations between anticitrullinated protein/peptide antibodies and rheumatoid factor. The value of the three anticitrullinated protein/peptide tests and of the RF test for predicting radiographic progression after five years was evaluated. After determination of the smallest detectable difference in radiograph scores, patients were separated into two groups, one with and one without progression. Progression of the total score, erosion score, and narrowing scores was calculated in patient subgroups defined by presence of anti-CCP ELISA antibodies, APF, AKA, or RF. The odds ratios (ORs) for significant radiographic deterioration were calculated, as well as the sensitivity and specificity of these autoantibodies in predicting Sharp score progression. Similar analyses were made after further stratification of patients according to RF status at baseline. We tested the hypothesis that patients with both RF and anti-CCP ELISA antibodies were more likely to show radiographic deterioration than patients with RF but without anti-CCP antibodies. ORs were calculated, and sensitivity and specificity for predicting Sharp score progression

Table 2 Performance of three anticitrullinated protein/peptide tests and of rheumatoid factor in predicting progression of the total, erosion, and joint space narrowing Sharp scores

Antibody positive at baseline	5 Year Sharp score	OR (95% CI)	Sensitivity	Specificity	PPV	NPV
Anti-CCP	Total	2.5 (1.2 to 5.0)	67	57	64	58
	Erosions	3.4 (1.6 to 7.2)	74	54	49	77
	Narrowing	1.8 (0.8 to 3.6)	62	51	56	58
APF	Total	2.4 (1.2 to 4.9)	57	64	66	64
	Erosions	1.3 (0.6 to 2.7)	52	55	35	71
	Narrowing	2.4 (1.2 to 4.7)	58	63	60	61
AKA	Total	1.3 (0.6 to 2.6)	38	68	57	50
	Erosions	1.2 (0.5 to 2.5)	32	64	29	67
	Narrowing	1.4 (0.7 to 2.8)	39	69	55	54
RF	Total	0.7 (0.3 to 1.5)	69	24	47	45
	Erosions	1.2 (0.5 to 2.8)	69	26	27	67
	Narrowing	0.5 (0.2 to 1.1)	65	22	40	45

OR, odds ratio; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; CCP, cyclic citrullinated peptides; APF, antiperinuclear factor; AKA, antikeratin antibody; RF, rheumatoid factor.

were determined for the three types of anticitrullinated protein/peptide antibodies.

Finally, mean Sharp score values (total score, erosion score, and narrowing score) were calculated for each group of patients with or without anticitrullinated protein/peptide antibodies. Then, comparisons of means at baseline and after three and five years were performed using the non-parametric Mann-Whitney test. Variations in Sharp scores between baseline, three years, and five years were also calculated. The variation from baseline to five years (Δ5 years) was termed the progression score; the mean value for this was calculated in all patients. The non-parametric Mann-Whitney test was used to compare patients with and without anti-CCP ELISA with and without APF, and with and without AKA. Values of $p<0.05$ were considered significant. Similar comparisons of mean Sharp score values were performed after further stratification of patients based on RF status at baseline and on anticitrullinated protein/peptide status. As anti-CCP ELISA test and Sharp's score are continuous quantitative variables, a linear correlation was determined between the Sharp total score or the Sharp progression score and anti-CCP units using a parametric Pearson's product-moment correlation coefficient.

RESULTS

Patient characteristics

At the time of the analysis, 156 patients had completed five years of follow up. Table 1 summarises the main characteristics of the cohort at baseline. After baseline sera were obtained, all patients received DMARD and non-steroidal anti-inflammatory drug treatment. During the first five years of

Table 3 Performance of three different anticitrullinated protein/peptide tests for predicting the total, erosion, and joint space narrowing Sharp scores. Patients were stratified according to baseline RF status

5 Year Sharp score	Antibody status at baseline		OR (95% CI)	Sensitivity	Specificity	PPV	NPV
Total	RF+	CCP+	2.3 (1 to 5.5)	67	54	61	59
		APF+	3.0 (1.3 to 7.2)	60	67	67	59
		AKA+	1.2 (0.5 to 2.7)	35	68	51	52
	RF–	CCP+	2.1 (0.5 to 8.3)	65	53	65	53
		APF+	1.4 (0.4 to 5.4)	53	56	59	50
		AKA+	1.3 (0.3 to 5.4)	40	67	61	45
Erosions	RF+	CCP+	2.5 (0.9 to 6.8)	72	49	37	81
		APF+	3.5 (1.3 to 9.2)	61	68	48	78
		AKA+	0.6 (0.2 to 1.6)	26	63	21	69
	RF–	CCP+	1.8 (0.4 to 7.8)	67	55	40	73
		APF+	0.7 (0.2 to 2.7)	42	48	29	61
		AKA+	2.3 (0.5 to 9.6)	50	69	44	73
Joint space narrowing	RF+	CCP+	2.2 (0.9 to 5.3)	68	52	53	67
		APF+	3.3 (1.4 to 7.7)	63	65	60	68
		AKA+	1.6 (0.7 to 3.8)	40	71	51	61
	RF–	CCP+	1.1 (0.3 to 4.4)	59	44	50	53
		APF+	1.4 (0.4 to 5.3)	53	55	53	55
		AKA+	2.3 (0.6 to 9.4)	48	72	61	59

OR, odds ratio; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; CCP, cyclic citrullinated peptides; APF, antiperinuclear factor; AKA, antikeratin antibody; RF, rheumatoid factor; +, positive; –, negative.

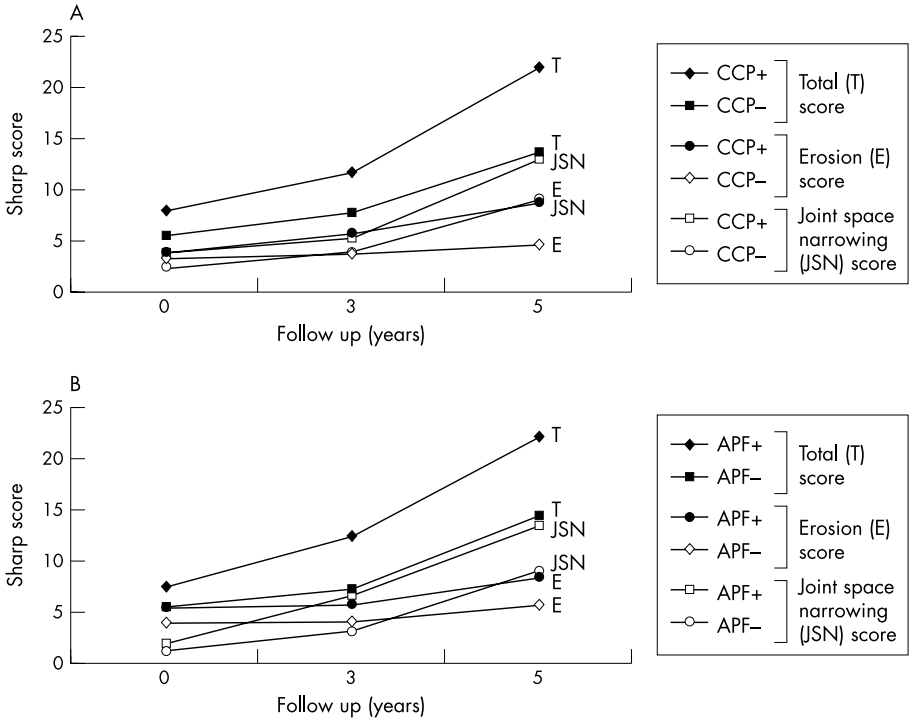

Figure 1 Mean (absolute) Sharp scores in patients with or without (A) anti-CCP ELISA antibodies or (B) APF antibodies, at baseline.

follow up, the mean number of DMARDS used by each patient was 1.95 (range 1–5), and most patients received methotrexate (191), salazopyrine (147), intramuscular gold (41), hydroxychloroquine (25), or D-penicillamine compounds (14). Eighty six patients (45%) received the same DMARD or the same combination of DMARDs throughout the five year follow up. Sixty three patients (33%) took at least one course of low dose (5–15 mg/day) prednisone treatment. Radiological data from 172 and 156 patients were available at three and five years.

Results of the anticitrullinated protein/peptide antibody tests were obtained for APF in 132 patients on baseline sera, for AKA in 145 patients, and for anti-CCP ELISA in 133 patients; 132 patients were tested with all three methods. Serum samples were obtained before DMARD treatment.

Comparison of the three anticitrullinated protein/peptide tests

At baseline, the anti-CCP ELISA was positive in 75/132 patients (sensitivity 57%), the APF IIF in 61/132 patients (sensitivity 46%), and the AKA IIF in 60/132 patients (sensitivity 45%). Twenty two of 71 sera negative for APF IIF were positive for anti-CCP ELISA (31%) and 8/58 sera negative for anti-CCP ELISA were positive for APF IIF (14%), yielding a 77% agreement rate between the two tests. Twenty three of 72 sera negative for AKA IIF were positive for anti-CCP ELISA (32%) and 8/60 sera positive for AKA IIF were negative for anti-CCP ELISA (13%), yielding an agreement rate of 76.5% between the two tests. At baseline, anticitrullinated protein/peptide antibodies were detected using at least one of the three methods in 87/130 (67%) patients tested with all three methods. For comparison IgM or IgA RF were present at baseline in 81% of the total cohort (table 1) and IgM RF in 90/130 (69%) patients tested with the three methods for anticitrullinated protein/peptide antibodies.

The frequencies of anti-CCP antibodies, APF, and AKA at baseline were similar in patients with and without RF: 57%, 48%, and 34%, respectively, in RF positive patients and 57%, 49%, and 37%, respectively, in RF negative patients (p non-significant for all three antifilaggrin antibodies).

Radiographic outcomes

After three years, the mean (SD) values for the total, erosion, and narrowing scores had increased from 3.6 (7.7) to 9.7 (13.9), from 1.7 (4.5) to 4.8 (7.3), and from 1.9 (3.7) to 4.9 (6.6), respectively. After five years of follow up, the total score had increased to 17.9 (22.3) ($p<0.001$), the erosion score to 6.9 (9.5) ($p<0.001$), and the narrowing score to 11.0 (15.4) ($p<0.001$). Median values of the total score were 1.0 at baseline, 4.0 at three years, and 10.0 at five years. Total score was 0 in 80 patients (42%) at baseline, 30 (17%) patients at three years, and 27 (17%) patients at five years. The patients without erosions were 111 (58%) at baseline, 42 (24%) after three years, and 35 (22%) after five years. Absence of narrowing was noted in 104 (54%) patients at baseline, 56 (32%) at three years, and 41 (26%) at five years. After three and five years, total score progression was found in 71 (41%) and 87 (56%) patients, respectively; erosion score progression in 55 (32%) and 75 (48%) patients, respectively; and narrowing score progression in 46 (27%) and 62 (40%) patients, respectively.

Predictors of radiographic damage

Among 133 patients tested for anti-CCP ELISA antibodies at baseline, 75 were positive. A significant increase in the total Sharp score after five years, defining progression, was seen in 72 of these patients. Anti-CCP antibodies were present at baseline in 48/72 patients with progression and in 27/61 patients without progression; sensitivity of anti-CCP ELISA for predicting total Sharp score progression was 67%, specificity was 56%, positive predictive value was 64%, and negative predictive value was 58%. The OR was 2.5 (95% CI 1.2 to 5.0). Table 2 shows a comparison of the sensitivity, specificity, and positive and negative predictive values of anticitrullinated protein/peptide antibodies and RF in predicting progression in Sharp scores (total, erosions, and narrowing) after five years, with the ORs and their 95% CIs. The ORs for significant Sharp score progression among patients with anti-CCP ELISA antibodies at baseline were 2.5 (95% CI 1.2 to 5.0) for the total score; 3.4 (95% CI 1.6 to 7.2) for the erosion score; and 1.8 (95% CI 0.8 to 3.6) for the narrowing score. Findings were similar for patients with a positive APF test at baseline: OR 2.4

Table 4 Sharp scores (absolute values) at baseline and after three and five years in patients with and without anti-CCP ELISA antibodies and with and without APF IIF

Follow up	Sharp score	Statistical difference between positive and negative for: Anti-CCP ELISA	APF IIF
Baseline	Total damage	0.0006	0.09
	Erosions	0.0004	0.051
	Joint space narrowing	0.032	0.14
3 years	Total damage	0.007	0.009
	Erosions	0.015	0.058
	Joint space narrowing	0.066	0.009
5 years	Total damage	0.001	0.008
	Erosions	<0.0001	0.004
	Joint space narrowing	0.029	0.010

(95% CI 1.2 to 4.9) for the total score; OR 1.3 (95% CI 0.6 to 2.7) for the erosion score; and OR 2.4 (95% CI 1.2 to 4.7) for the narrowing score. No significant increase in the OR for radiographic progression at five years was seen in patients with a positive AKA test or RF ELISA at baseline.

Patients were further classified according to RF baseline status and type of baseline anticitrullinated protein/peptide antibodies. Patients with RF were more likely to develop joint damage if they were also positive for anti-CCP ELISA antibodies (total Sharp score, table 3). For example, among the 86 RF positive patients at baseline, 49 were positive and 37 were negative for anti-CCP ELISA. Of these 86 patients, 45 had radiographic progression. Total Sharp score progression was seen in 30/49 (61%) anti-CCP-ELISA positive patients and 15/37 (41%) anti-CCP-ELISA negative patients ($p=0.05$). The OR for total Sharp score progression in patients with both RF and anti-CCP ELISA at baseline was 2.3 (95% CI 1 to 5.5). Similar data were obtained for patients with RF and APF at baseline but not for patients with RF and AKA at baseline. Table 3 summarises these data.

Sharp scores (mean and ranges) at baseline and after three and five years were calculated in the patients with and without anti-CCP ELISA antibodies, with and without APF, and with and without AKA (table 4). Figure 1 reports absolute values of the mean total, erosion, and narrowing scores at the three times. Table 5 shows differences between baseline and three or five years and table 6 the values of these scores in patients with anti-CCP ELISA, APF IIF, and/or AKA IIF further stratified according to RF status at baseline.

Mean total score and mean erosion score differed significantly between patients with and without anti-CCP ELISA antibodies at baseline. The difference was still highly significant after three and five years of follow up (fig 1A).

Anti-CCP ELISA status also had a highly significant influence on radiographic score worsening between baseline and three or five years: the total score, erosion score, and narrowing score were higher in the patients with than without anti-CCP ELISA antibodies (table 5).

Similar differences were found between patients with and without APF IIF antibodies: the former had higher radiographic scores at baseline and after three and five years of follow up (fig 1B).

No differences were noted between patients with and without AKA IIF antibodies, even after five years of follow up (data not shown).

Patients were stratified according to RF status, and mean radiographic scores in RF positive patients were compared with those in RF negative patients with or without the three anticitrullinated protein/peptide antibodies (table 6). At baseline, RF positive patients with anti-CCP ELISA antibodies had a significantly higher mean erosion score than RF positive patients without anti-CCP ELISA antibodies (4.4 *v* 3.1, $p<0.03$). Similarly, the mean erosion score was significantly higher in RF negative patients with anti-CCP ELISA antibodies than in RF negative patients without anti-CCP ELISA antibodies (9.6 *v* 3.1, $p<0.02$). After three years of follow up, mean total score was higher in RF positive patients with than without anti-CCP ELISA antibodies (6.4 *v* 5.8, $p<0.05$). Findings in RF positive patients were similar for the total and erosion scores at baseline in patients with and without APF IIF antibodies—for example, the mean total scores were 7.1 and 3.8 ($p<0.04$) and the mean erosion scores were 5.3 and 2.5 ($p<0.02$) in these two groups at baseline, respectively. After three years of follow up, the mean total, erosion, and narrowing scores were higher in RF positive patients with APF IIF antibodies than in RF positive patients without APF IIF antibodies (data not shown).

Similar data were found after five years of follow up (table 6). The mean total score was higher and score progression during the five year follow up was more severe in RF negative patients with anti-CCP antibodies than in RF positive patients without anti-CCP antibodies. Data were similar after five years for APF IIF antibodies (table 6) and remained non-significant for AKA IIF antibodies (data not shown).

Anti-CCP antibody concentration was not correlated with the *x* ray damage determined using Sharp's total score at five years (absolute score: $p<0.2$ or progression score: $p<0.08$).

DISCUSSION

The objective of this study was to assess the value of three anticitrullinated protein/peptide antibody tests in predicting

Table 5 Variation in Sharp score values (mean and range) between baseline and three and five years in patients with rheumatoid arthritis with or without anticitrullinated protein/peptide autoantibodies. Results are shown as mean (range)

Baseline status	Progression 0–3 years: Total	Erosions	Joint space narrowing	Progression 0–5 years: Total	Erosions	Joint space narrowing
Anti-CCP+	11.98 (0–72)	6.11 (0–46)	5.85 (0–48)	16.46 (0–89)	6.04 (0–28)	10.43 (0–67)
Anti-CCP–	7.32 (0–55)	3.44 (0–30)	3.87 (0–33.5)	8.60 (0–77)	3.41 (0–46)	5.19 (0–36)
p	0.0006	0.0004	0.032	0.002	<0.0001	0.0049
APF +	9.34 (0–58)	4.6 (0–38)	4.74 (0–44)	17.15 (0–89)	6.06 (0–28)	11.09 (0–67)
APF –	5.08 (0–39)	3.19 (0–29)	1.72 (0–18)	9.68 (0–77)	4.02 (0–46)	5.65 (0–53)
p	0.009	0.058	0.009	0.0072	0.0089	0.0011
AKA +	7.81 (0–57)	3.96 (0–38)	3.72 (0–22)	14.0 (0–73)	4.74 (0–28)	9.25 (0–62)
AKA –	6.86 (0–58)	3.85 (0–22)	2.93 (0–44)	12.78 (0–89)	5.13 (0–46)	7.64 (0–67)
p	0.22	0.17	0.50	0.21	0.37	0.19

CCP, cyclic citrullinated peptides; APF, antiperinuclear factor; AKA, antikeratin antibody.

Table 6 Progression of Sharp scores from baseline to three and five years in patients with or without rheumatoid factor and with or without anticitrullinated proteins/peptides antibodies

	3 Years			5 Years		
	Mean total score	Erosions	Joint space narrowing	Mean total score	Erosions	Joint spacing narrowing
RF+ CCP+	6.4	3.3	2.9	14.5	5.3	9.1
	p = 0.047*			p = 0.002	p = 0.0005	p = 0.02
RF+ CCP–	5.8	3.0	2.6	7.8	3.0	4.7
				p = 0.01	p = 0.006	p = 0.044
RF– CCP+	11.5	6.7	4.6	19.8	8.3	11.5
RF– CCP–	7.8	5.2	2.5	10.6	4.3	6.3
RF+ APF+	7.9	4.2	3.8	15.4	5.7	9.7
	p = 0.009	p = 0.02	p = 0.03	p = 0.003	p = 0.01	p = 0.006
RF+ APF–	4.2	2.3	1.7	8.7	3.4	5.4
						p = 0.04
RF– APF+	10.8	5.7	5.0	18.6	6.3	11.2
RF– APF–	7.8	5.6	2.1	14.0	6.3	7.4

*Only p values <0.05 are reported.
CCP, cyclic citrullinated peptides; APF, antiperinuclear factor; AKA, antikeratin antibody.

radiographic outcomes in patients with recent onset RA. Two of the three tests, APF and AKA, were IIF assays, and the remaining test was an ELISA that detects antibodies to CCP. All three tests detect autoantibodies to modified linear proteins derived from profilaggrin. Citrullination of these proteins results from deimination of arginine residues.[8 10] We tested the value of these antibodies determined at baseline (within 12 months of symptom onset) for predicting joint damage three and five years later.

The first important finding from our study is that the three tests differed in their sensitivity for early RA: at baseline, AKA was the least sensitive test (45%), APF was intermediate (46%), and anti-CCP ELISA was the most sensitive test (57%). These data are in accordance with previous publications on serological tests in early RA: for instance, Van Jaarsveld and coworkers found APF in 66% and anti-CCP ELISA in 52% of 249 patients with early RA.[24] The discrepancies between the three tests can be explained by the differences between the substrates used for determination of anticitrullinated proteins.

The value of autoantibodies for predicting RA outcomes is still a matter of debate. Abundant data indicate, however, that long term joint destruction is more severe in patients with IgM RF than in those without RF,[13 15–19 31–35] although substantial differences exist across studies.

Radiographic damage, analysed through the measurement of the Sharp score modified by van der Heijde, progresses at stable rates during the course of RA. The mean rate of modified Sharp unit per year has been measured during recent inception cohort studies of RA: 8.6 for the total score, 5.4 for the erosion score, and 3.2 for the narrowing score[36] and even more in a second cohort studies: 10.9 for the total score.[37]

Among anticitrullinated protein/peptide antibodies, APF was associated with more severe joint damage in cohort studies of patients with longstanding RA[21 25] and in a cross sectional study of patients with destructive or non-destructive RA.[13]

The value of anticitrullinated protein/peptide antibodies for predicting RA outcomes has been investigated only recently in prospective cohorts of patients with early RA.[24 38] Our data show that the proportion of patients with significant five years *x* ray progression of joint destruction was higher among patients with anti-CCP ELISA or APF at baseline than in patients without: 67% *v* 44% for anti-CCP antibodies; 67% *v* 49% for APF. Our data show clearly that anti-CCP ELISA and APF antibodies (but not AKA antibodies) are equivalent in predicting joint damage assessed by Sharp scores. The total damage score and its components, the erosion score and the narrowing score, were more severe in patients with than in those without anti-CCP ELISA or APF antibodies.

The total damage score and the erosion score at baseline—that is, within 12 months after symptom onset, were significantly higher in the patients with anti-CCP ELISA and/or APF antibodies.

After three and five years, radiographic joint damage was significantly more severe in the patients with anti-CCP ELISA and/or APF antibodies. Because RF status may be associated with the risk of joint damage, we compared RF positive and RF negative patients. Among RF positive patients, those with APF had higher mean Sharp scores than those without APF, both at baseline and after three or five years. After five years, RF negative patients with anti-CCP ELISA had more severe joint damage than RF positive patients without anti-CCP ELISA. In contrast with the study of Kroot *et al*,[38] we found that RF negative patients with anti-CCP ELISA had significantly more severe joint damage after five years than RF negative patients without anti-CCP ELISA. The same was true for the narrowing score in patients with and without APF.

Our data suggest that anti-CCP ELISA or APF IIF at RA onset may be better predictors of joint damage than RF. Similar data have been obtained by Van Jaarsveld *et al* in a series of 249 patients with early RA followed up for three years.[24] However, Paimela *et al* reported that a positive ELISA based on purified human filaggrin as the antigen failed to predict joint outcomes.[39]

AKA tested by IIF did not predict radiographic progression during our five year follow up. In a study of patients with RA, Paimela and coworkers found a non-significant trend towards radiographic progression in 51% of patients with RA with AKA compared with 36% of patients with RA without AKA.[23] Similar findings have been reported by Forslind *et al*.[40] The low prevalence of AKA in early RA might explain why this autoantibody does not have prognostic value.

Other factors that probably have a substantial influence on joint outcomes in RA include HLA DRB1* alleles, marked increases in laboratory parameters for inflammation (ESR and

CRP), and baseline joint damage. In a previous study,[41] a multiparameter prospective analysis using logistic regression analysis has defined an arithmetic score that could be used to predict radiographic damage and radiographic progression in individual patients. However, our findings indicate that a simple inexpensive test such as anti-CCP ELISA (or APF IIF) combined with RF determination is useful in predicting severe joint destruction during the first five years after onset of RA.

Authors' affiliations
O Meyer, Service de Rhumatologie, CHU Bichat, AP-HP, Paris, France
C Labarre, P Nicaise-Roland, Service d'Immunologie et Sérologie, Bichat, AP-HP, Paris, France
M Dougados, Service de Rhumatologie, CHU Cochin, AP-HP, Paris, France
Ph Goupille, Service de Rhumatologie, CHU Tours, France
A Cantagrel, Service de Rhumatologie, CHU Rangueil, Toulouse, France
A Dubois, B Combe, Fédération de Rhumatologie, CHU Montpellier, France
J Sibilia, Service de Rhumatologie, CHU Strasbourg, France
C Labarre, Laboratoire d'Immunologie, Faculté de Pharmacie, Université Paris XI, France

REFERENCES

1 **Berthelot JM**, Maugars Y, Castagne A, Audrain M, Prost A. Antiperinuclear factors are present in polyarthritis before ACR criteria for rheumatoid arthritis are fulfilled. Ann Rheum Dis 1997;56:123–5.
2 **Nienhuis RLF**, Mandema E. A new serum factor in patients with rheumatoid arthritis, the antiperinuclear factor. Ann Rheum Dis 1964;23:302–5.
3 **Hoet RM**, van Venrooij WJ. The antiperinuclear factor (APF) and antikeratin antibodies (AKA) in rheumatoid arthritis. In: Smolen JS, Kalden JR, Maini RN, eds. *Rheumatoid arthritis*. Berlin, Heidelberg: Springer, 1992:299–318.
4 **Cordonnier CC**, Meyer O, Palazzo E, de Bandt M, Elias A, Nicaise P, *et al*. Diagnostic value of anti-RA33 antibody, antikeratin antibody, antiperinuclear factor and antinuclear antibody in early rheumatoid arthritis: comparison with rheumatoid factor. Br J Rheumatol 1996;35:620–4.
5 **Goldbach Mansky R**, Lee J, McCoy A, Hoxworth J, Yarboro C, Smolen JS, *et al*. Rheumatoid arthritis-associated autoantibodies in patients with synovitis of recent onset. Arthritis Res 2000;2:236–43.
6 **Aho K**, Von Essen R, Kurki P, Palosuo T, Heliovaara M. Antikeratin antibody and antiperinuclear factor as markers for subclinical rheumatoid disease process. J Rheumatol 1993;20:1278–81.
7 **Young BJJ**, Mallya RK, Leslie RDG, Clark CJM, Hamblin TJ. Antikeratin antibodies in rheumatoid arthritis. BMJ 1979;ii:97–9.
8 **Schellekens GA**, de Jong BAW, van den HOOGEN FHJ, van de Putte LBA. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis specific autoantibodies. J Clin Invest 1998;101:273–81.
9 **Girbal-Neuhauser E**, Durieux JJ, Arnaud M, Dalbon P, Sebbag M, Vincent C, *et al*. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. J Immunol 1999;162:585–94.
10 **Masson-Bessiere C**, Sebbag M, Girbal-Neuhauser E, Nogueira L, Vincent C, Senshu T, *et al*. The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the α and β chains of fibrin. J Immunol 2001;166:4177–84.
11 **Menard HA**, Lapointe E, Rochdi MD, Zhou ZJ. Insights into rheumatoid arthritis derived from the Sa immune system. Arthritis Res 2000;2:429–32.
12 **Schellekens GA**, Visser H, de Jong BAW, van den Hoogen HJ, Hazes JNW, Breedveld FC, *et al*. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155–63.
13 **Combe B**, Eliaou JF, Daurès JP, Meyer O, Clot J, Sany J. Prognostic factors in rheumatoid arthritis. Comparative study of two subsets of patients according to severity of articular damage. Br J Rheumatol 1995;34:529–34.
14 **Van der Heijde DMFM**, van Riel PLCM, van Leuween MA, van't Hof MA, van Rijswijk MH, van de Putte LBA. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis: a prospective follow-up study of 147 patients. Br J Rheumatol 1992;31:519–25.
15 **Young A**, Corbett M, Winfield J, Jagueremada D, Williams P, Papasawas G, *et al*. A prognostic index for erosive changes in the hands, feet and cervical spines in early rheumatoid arthritis. Br J Rheumatol 1988;27:94–101.
16 **Feigenbaum SL**, Masi AT, Kaplan SB. Prognosis in rheumatoid arthritis. A longitudinal study of newly diagnosed younger adult patients. Am J Med 1979;66:377–84.
17 **Kaarela K**. Prognostic factors and diagnostic criteria in early rheumatoid arthritis. Scand J Rheumatol Suppl 1985;57:5–54.
18 **Van Zeben D**, Hazes JMW, Zwinderman AH, Vandenbroucke JP, Breedveld FC. Factors predicting outcome of rheumatoid arthritis: results of a follow-up study. J Rheumatol 1993;20:1288–96.
19 **Mottonen T**, Paimela L, Leirisalo-Repo M, Kautiainen H, Ilonen J, Hannonen P. Only high disease activity and positive rheumatoid factor indicate poor prognosis in patients with early rheumatoid arthritis treated with "sawtooth" strategy. Ann Rheum Dis 1998;57:533–9.
20 **Von Essen R**, Kurki P, Isomaki H, Okubo S, Autiainen H, Aho K. Prospect for an additional laboratory criterion for rheumatoid arthritis. Scand J Rheumatol 1993;22:267–72.
21 **Munos Fernandes S**, Alvarez Doforno R, Gonzales Tarrio JM, Balso A, Richi P, Fontan G, *et al*. Antiperinuclear factor as a prognostic marker in rheumatoid arthritis. J Rheumatol 1999;26:2572–7.
22 **Janssens X**, Veys EM, Verbruggen G, Declercq L. The diagnostic significance of the antiperinuclear factor for rheumatoid arthritis. J Rheumatol 1988;15:1346–50.
23 **Paimela L**, Gripenberg M, Kurki P, Leirisalo-Repo M. Antikeratin antibodies: diagnostic and prognostic markers for early rheumatoid arthritis. Ann Rheum Dis 1992;51:743–6.
24 **Van Jaarsveld CHM**, ter Borg EJ, Jacobs JWG, Schellekens GA, Gmelig-Meyling FHJ, van Booma-Frankfort C, *et al*. The prognostic value of the antiperinuclear factor, anti-citrullinated peptide antibodies and rheumatoid factor in early rheumatoid arthritis. Clin Exp Rheumatol 1999;17:689–97.
25 **Westgeest AA**, Boerbooms AM, Jongmans M, Vandenbroucke JP, Vierwinden G, van de Putte LB. Antiperinuclear factor: indicator of more severe disease in seronegative rheumatoid arthritis. J Rheumatol 1987;14:893–7.
26 **Bas S**, Perneger TV, Mikhnevitch E, Seitz M, Tiercy JM, Roux Lombard P, *et al*. Association of rheumatoid factors and anti-filaggrin antibodies with severity of erosions in rheumatoid arthritis. Rheumatology (Oxford) 2000;39:1082–8.
27 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
28 **Bland JM**, Altman DC. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:1036–8.
29 **Lassere M**, Boers M, van der Heijde D, Boonen A, Edmonds J, Saudan A, *et al*. Smallest detectable difference in radiological progression. J Rheumatol 1999;26:731–9.
30 **Meyer O**, Tauxe F, Fabregas D, Gabay C, Goycochea M, Haim T, *et al*. Anti-RA 33 antinuclear autoantibody in rheumatoid arthritis and mixed connective tissue disease: comparison with antikeratin and antiperinuclear antibodies. Clin Exp Rheumatol 1993;11:473–8.
31 **Van der Heidje DM**, van Riel PL, Nuver Zwart HH, Gribnau FW, van de Putte LBA. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet 1989;i:1036–8.
32 **Van Zeben D**, Breedveld FC. Prognostic factors in rheumatoid arthritis. J Rheumatol 1996;23(suppl. 44):31–3.
33 **Van der Heide A**, Remme CA, Hofman DM, Jacobs JWG, Bijlsma JWJ. Prediction of progression of radiologic damage in newly diagnosed rheumatoid arthritis. Arthritis Rheum 1995;38:1466–74.
34 **Van Zeben D**, Hazes JMW, Zwinderman AH, Cats A, van der Voor EAM, Breedveld FC. Clinical significance of rheumatoid factors in early rheumatoid arthritis: results of a follow-up study. Ann Rheum Dis, 1992;51:1029–35.
35 **Möttönen TT**, Hannonen P, Arvilommi M, Jokinen I, Oka M. Relation between bone erosions and rheumatoid factor IgA and IgM isotypes in recent onset rheumatoid arthritis. Scand J Rheumatol Suppl 1988;75:244–9.
36 **Hulsmans HMJ**, Jacobs JWG, van der Heijde DMFM, van Albada-Kuipers GA, Schenk Y, Bijlsma WJ. The course of radiologic damage during the first six years of rheumatoid arthritis. Arthritis Rheum 2000;43:1927–40.
37 **Drossaers-Bakker KW**, Amesz E, Zwinderman AH, Breedveld FC, Hazes JMW. Relationship between time-integrated C-reactive protein levels and radiologic progression in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:1465–72.
38 **Kroot EJJA**, de Jong BAW, van Leeuwen MA, Swinkels H, van den Hoogen FHJ, van't HOF M, *et al*. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831–5.
39 **Paimela L**, Palosuo T, Aho K, Lukka M, Kurki P, Leirasalo-Repo M, *et al*. Association of autoantibodies to filaggrin with an active disease in early rheumatoid arthritis. Ann Rheum Dis 2001;60:32–5.
40 **Forslind K**, Vincent C, Serre G, Svensson B. Antifilaggrin autoantibodies in early rheumatoid arthritis. Scand J Rheumatol 2000;29:320–2.
41 **Combe B**, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J, *et al*. Prognostic factors for radiographic damage in early rheumatoid arthritis. A multiparameter prospective study. Arthritis Rheum 2001;44:1736–43.